REMARKS

Claims 1-103, 126-130, and 138-143 have been cancelled. Claims 121-125 and 131-137 have been withdrawn. Claims 104, 144, 225, and 232 have been amended. Claims 237 and 238 are newly added. No new matter has been added.

Rejections Under 35 U.S.C. §112, second paragraph

The Examiner rejected claims 104-108, 113, 119, 144-148 and 225-236 under 35 U.S.C. §112, second paragraph as allegedly indefinite. The Examiner argued that the claims do not recite a positive process step that refers back to the preamble of the claim, e.g., a step whereby the polyketide is produced.

Claims 104, 144, 225 and 232 have been amended to recite a positive process step referring back to the claim preamble. For example, claim 104 has been amended to include the phrase "whereby production of the polyketide is modulated". The remaining pending claims depend from one or another of claims 104, 144, 225 and 232. In view of the forgoing, Applicants respectfully request that the rejections under 35 U.S.C.§112, second paragraph be withdrawn.

Rejections Under 35 U.S.C. §112, first paragraph (enablement)

The Examiner rejected claims 104-108, 113, 119, 144-148 and 225-236 under 35 U.S.C. 112, first paragraph.

The Examiner argued that the claims are not enabled because, according to the Examiner: 1) the claims encompass the "production of a large number of polyketides, many of which are not produced by fungi because the specific enzymes required for their production ... are not present in the organism"; 2) the "state of the art clearly indicates that the metabolic engineering of strains to produce polyketides is very demanding in that it requires the functional expression of a large number of enzymes for each type or polyketide, the presence of appropriate amounts of precursors for their production, as well as a the ability of the host cell to produce large

quantities of the desured polyketide without toxicity to the host cell; and 3) the specification "provides no guidance or working examples as regards the use of *CreA*".

The present claims are drawn to methods for modulating the production of a polyketide produced by a fungal cell by overexpressing or conditionally expressing CreA in the fungal cell and to methods for producing a polyketide produced by a fungal cell by overexpressing or conditionally expressing CreA in the fungal cell. The fungal cells of the claims are ones which already produce the polyketide, even in the absence of *CreA*, e.g., because they naturally produce the polyketide or have been modified to do so. The overexpression or conditional expression of CreA does not itself confer the ability to produce CreA to the fungal cell. As the present specification notes, CreA is a transcription factor that modulates the expression of gene involved in carbon metabolism (see page 14 of the specification). Applicants have amended claims 104, 144, 225 232 to clarify that the fungal cell is one which produces the polyketide.

Because the fungal cell is one which produces the polyketide, the Examiner's concerns regarding the presence or absence of enzymes required for the biosynthesis of the polyketide, the availability of precursors, and the possible toxicity of the a given polyketide to the fungal cell are misplaced and simply irrelevant. It is true that Pfeifer et al. (*Microbiology and Molecular Biology Reviews* 65:106, 2001), cited by the Examiner, discusses the challenges associated with engineering cells to produce polyketides. However, the very title of this article, Biosynthesis of Polyketides in Heterologous Hosts, makes it clear that the primary concern of the authors is the production of a polyketide in a cell that does not otherwise produce the polyketide. This is not the context of the present claims. Moreover, Pfeifer et al. is concerned with production of polyketides in diverse species, including *E. coli* and other bacteria as well as plants. In contrast, the present claims are limited to production of polyketides in fungal cells. With regard to filamentous fungi, Pfiefer et al. note that they are "prolific producers of polyketides". Thus, Pfeifer et al. cannot be seen as support for the Examiner's assertion that the claims are not enabled.

Turning to the Examiner's contention that the specification provides "no guidance or working examples as regards the use of *CreA*", it is Applicants' position that those skilled in the

art are capable of practicing the claimed methods. The CreA gene is known in the art as are vectors for overexpressing or conditionally expressing a gene of interest in a fungal cell. As for methods for culturing cells to produce polyketides, the culturing of fungal cells to produce polyketides has long been known in the art. For example, U.S. Patent 5,849,541 describes methods for culturing *A. terreus* to produce lovastatin. Moreover, the specification, in Example 2, describes method for culturing *A. terreus* under conditions that lead to the production of the polyketide, lovastatin.

As explained in greater detail in the attached Declaration of Edward Driggers Under 37 C.F.R. §1.132, both *A. terreus* and *P. citrinum* transformed with a CreA expression exhibit altered polyketide production.

First, transformation of *P. citrinum* with a CreA expression vector increased compactin production compared to control *P. citrinum* transformed with an empty vector not expressing CreA (see paragraphs 10-13 of the Declaration of Edward Driggers Under 37 C.F.R. §1.132).

Second, transformation of *A. terreus* with a CreA expression vector increased geodin production 2.6-fold and lovastain production 4.16-fold compared to control *A. terreus* transformed with an empty vector not expressing CreA (see paragraph 9 of the Declaration of Edward Driggers Under 37 C.F.R. §1.132).

Finally, as shown in the table below, transformation of *A. terreus* with a CreA expression vector modulated production of eight different polyketides compared to control non-tranformed *A. terreus* (see paragraphs 3-8 of the Declaration of Edward Driggers Under 37 C.F.R. §1.132). As shown in the table below, copied from the Declaration of Edward Driggers Under 37 C.F.R. §1.132, production of six polyketides was increased and production of two polyketides was decreased in cells transformed with CreA. In the context of producing a given polykektide it is, of course, useful to increase polykektide production. However, it can also be useful to decrease production of a given polyketide, for example to conserve resources that could be used by the cell for production of a different polyketide.

Polyketide	**Polyketide production ratio in CreA transformed cells relative to non-transformed cells** **P value**	**Is this polyketide part of a statin pathway?**	**CAS registry number**	**Reference number**
3,5-Dihydro-3-hydroxymonacolin L	6.8 ($p=7.7 \times 10^{-10}$)	yes	119786-66-2	1
Acetyl Lovastatin, closed	7.6 ($p=7.3 \times 10^{-7}$)	yes	Not available	2
Erdin; (%)-form 7-Dechloro, Me ester	0.46 ($p=0.0137$)	no	103470-59-3	3
Erdin; (R)-form (lacking the methylester on the bottom)	4.2 ($p=9.73 \times 10^{-5}$)	no	26891-81-6	4
Mevinolin; 4,4a-Dihydro	5.5 ($p=7.99 \times 10^{-8}$)	yes	77517-29-4	5
Monacolin J; 1-Deoxy	7.2 ($p=1.29 \times 10^{-9}$)	yes	79394-47-1	6
Osoic acid; 3-Me ether, 1-Me ester	0.48 ($p=0.00334$)	no	577-64-0	7
Tiglistatin, open	>10*	yes	Not available	8

*The production of tiglistatin by non-transformed cells was too low to be detected, but based on the apparent lower limit of detection in this assay it was estimated that CreA transformed cells express at least 10-fold more tiglistatin than non-transformed cells.

The studies described in the Edward Driggers Under 37 C.F.R. §1.132 demonstrate that transformation of two different fungal species with a CreA expression vector modulated polyketide production, supporting Applicants' position that the present claims are enabled.

Applicants note that the Examiner argued that a previously submitted declaration under 37 C.F.R. §1.132 did not provide evidence of enablement because the vectors and culture conditions used were not described in the specification. This is not a proper standard for assessing enablement. "A patent must contain a description that enables one skilled in the art to

make and use the claimed invention....An inventor need not, however, explain every detail since he is speaking to those skilled in the art." *DeGeorge v. Bernier* 768 F.2d 1318 (Fed. Cir. 1985). As noted above, CreA was known in the art well prior to the priority date of the present application as were methods for expressing a selected gene in a fungal cell using expression vectors. As for the culture conditions, as recited in the declaration, the culture conditions for producing the polyketides were known in the prior art. Moreover, the specification in Example 2 describes culture conditions for the production of lovastatin by *A. terreus*. Thus, the claims are enabled irrespective of whether the specification teaches the vectors and culture conditions of the previously submitted declaration under 37 C.F.R. §1.132 or the Declaration of Edward Driggers Under 37 C.F.R. §1.132 are disclosed in the present specification.

In view of the forgoing, Applicants respectfully request that the rejections under 35 U.S.C.§112, second paragraph be withdrawn.

Enclosed is a $475.00 check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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